

# Macroinvertebrate abundance and diversity in two ten-year-old created wetlands

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## Abstract

Macroinvertebrates were sampled in two experimental wetlands in Columbus, OH, ten years after the wetlands were created, and the data were compared to data from previous years. One wetland was planted 10 years prior to the sampling while the other wetland was allowed to colonize naturally. The 10-year-old wetlands were sampled twice: a broad sampling in September 2004 using three methods: Hester-Dendy colonization plates, bottle traps, and dipnets; and sampling done annually in October 2004 using only Hester-Dendy plates. The September sampling collected a combined total of 35 taxa using the three different methods. Hester-Dendy sampling alone collected 24 taxa between the two wetlands in September, while the October sampling collected a total of 19 taxa over a 15-day period. The Shannon-Wiener diversity index ( $H'$ ) was 1.82 for the planted wetland (evenness = 0.60) and 2.22 for the naturally colonizing wetland (evenness = 0.73) in September, while in October it was reversed with 2.06 for the planted wetland (evenness = 0.73) and 1.58 for the naturally colonizing wetland (evenness = 0.62). Changes in algal abundance between the two wetlands in the fall and the decrease in temperature as fall progressed may explain the reversal of the indices. The wetlands have shown a general trend of increased diversity over time, with higher numbers of terrestrial species captured in the most recent surveys, possibly indicating increased availability of food and habitat for macroinvertebrates in the two wetlands as they mature.

## Introduction

Benthic macroinvertebrates are an important ecological component of wetland ecosystems and include annelid worms, mollusks, water mites, crustaceans and insects. The term “benthic” refers to bottom-living organisms, which live in the aquatic sediment for at least part of their life cycle. The “macro” in macroinvertebrates generally refers to invertebrates that are large enough to be seen with the unaided eye (Voshell, 2002), or to be retained by a standard sieve mesh opening size, usually about 500 microns (McCafferty and Provonsha, 1981). However, there has been a general trend among stream ecologists to use a finer mesh (e.g. 125 to 250 microns) in order to collect early life stages of macroinvertebrates that would pass through larger openings (Hauer and Resh, 1996). A smaller-size mesh

would also include some microinvertebrates, an addition that has been recommended by Halse et al. (2002). The September sampling period of this study used a sieve with a mesh size of 420 microns.

Communities of aquatic organisms respond to changes in their environment such as water quality. They reveal longer-range trends than water samples alone, which may reflect the quality of water only at the time the sample was taken. Periodic sampling of aquatic organisms is a tool to measure the condition of aquatic ecosystems over time. Good water quality is generally characterized by a diverse benthic fauna, without excessively large numbers of any one group (Geological Survey, 1977; Greeson et al., 1977). A combination of oxygen-rich open water and detritus-producing emergent plant areas has been found to maximize invertebrate biodiversity (Nelson et al., 2000).

Macroinvertebrates are the most commonly chosen group of freshwater organisms used for biomonitoring, since they are readily sampled, their many species result in a wide range of responses to environmental change, and some kinds are very sensitive to stress from pollution and habitat modification (Chutter, 1995; Voshell, 2002). Limitations of using macroinvertebrates for biomonitoring include the facts that they are not readily quantitatively sampled, that they often have seasonal variation in their occurrence, that species may drift into areas where they are not normally found, and that they are often difficult to identify (Rosenberg and Resh, 1993; Chutter, 1995).

Wetlands can support diverse invertebrate communities including aquatic, semiaquatic and terrestrial species. Some invertebrates, such as certain mosquitoes and fairy shrimp, are obliged to live in wetlands while a number of terrestrial insects including some beetles, moths and butterflies feed only on wetland plants. In addition, many invertebrates that evolved in other habitats can opportunistically inhabit wetlands (Sharitz and Batzer, 1999).

Aquatic insects are important food items, especially in wetland food webs where the survival of fish, amphibians and birds depends on them (McCafferty and Provonsha, 1981). Waterfowl require a high-protein diet, especially nesting hens and early-stage juveniles, and may feed exclusively on aquatic invertebrates during those life-stages (Swanson and Duebbert, 1989; Batzer and Wissinger 1996).

In addition to food web support, aquatic invertebrates may support other ecosystem functions such as nutrient cycling and the maintenance of water quality from filter-feeders'

Table 1. Summary of benthic macroinvertebrate studies at the Olentangy River Wetland Research Park

Year of study	Author(s)	Dominant taxa	#Organisms	#Taxa	Methods*	When sampled
1994	Nairn et al. (1995)	Gastropods	772	10	Colonization plates plus surber	Oct. 7- 28
1994	Dabrowska and Lentz (1995)	Mollusks	NA	9	Ceramic tile colonization plates	Dec.
1994	Martin and Armitage (1995)	NA	NA	16	Sieve and forceps	Sept.- Nov.
1994	Minamyer (1995)	Odonates only	NA	18	Seine (part of fish survey) and aerial nets	June
1995	Dabrowska (1996)	Chironomidae	NA	18	Colonization plates	Oct-Nov.
1995	Metzker (1996)	Chironomidae	1883	25	Clay colonization plates	Oct-Nov.
1996	Hart et al. (1996)	Gastropods	3225	38 in W1 and 32 in W2	DN, HD and clay colonization	Oct. 10-24
1996	Dabrowska (1997)	Gastropods	NA	20	Substrate colonization plates	Oct. - Nov.
1997	Spieles (1998); Spieles and Mitsch (2000)	Chironomidae	1557	41	HD, ET	Apr. - Oct.
1997	Cochran (1998)	Gastropods	1907	9	HD, minnow traps	Oct. 6 - Nov. 3
1998	Lowry (1999)	Gastropods	1355	10	HD, BT, DN	Oct.
1998	Custer and Johnson (1999)	Gastropods	1355	19	BT, HD, DN	Oct.
1999	Frazier and Mitsch (2000)	Gastropods	NA	8	HD, ET, FT	Oct.
1999	Custer et al. (2000)	Chironomidae	264	36	HD, ET	Apr. 2 - May 6
2000	Acharyya and Mitsch (2001)	Gastropods	572	15	HD,DN, BT	Oct.
2001	Webb and Mitsch (2002)	Gastropods	510	12	HD, DN	Oct.
2002	Holland and Mitsch (2003)	Collectors in outflow	894	19	HD, BT	Oct.
2003	Grubh and Mitsch (2004)	Oligochaetes in HD; Gastropods in DN	22880	26	HD, DN	Oct 9-Nov. 6
2004a	Current study	Gastropods overall	4895	35	HD,BT,DN	Sept.
2004b	Current study	Amphipods	1112	19	HD	Oct.

\* HD = Hester-Dendy colonization plate; BT = Bottle trap, DN = Dipnet, FT = Funnel trap

removal of algae (Duffy, 1999).

The potential success of higher-level consumers can be predicted by the quantity and diversity of macroinvertebrates present, because fish and other lower-level consumers feed on macroinvertebrates, and are themselves eaten by birds and other higher-level consumers (Lodge, 1994).

This study examined macroinvertebrates in two created wetlands in Ohio, USA. These riparian marshes provide a special opportunity to study the development of macroinvertebrate communities over time in newly created wetlands. The objectives of this study were to a) determine the abundance and diversity of macroinvertebrates in the two created wetlands ten years after their construction; b) compare diversity and abundance between the wetlands (one was planted at the time of construction while the other was allowed to colonize naturally) and between the inflow, middle, and outflow regions of the wetlands; c) compare taxa collected using three different methods; and d) compare results with previous years' studies in the same created wetlands.

## Methods

### Study Area

The study area consisted of planted Wetland 1 (W1) and naturally colonizing Wetland 2 (W2), at the Olentangy River Wetland Research Park, on the Ohio State University campus in Columbus, Ohio, adjacent to the Olentangy River. The two 1-ha perched wetlands were created in 1994 as a full-scale experiment in which Wetland 1 was planted with twelve common wetland species while Wetland 2 was left unplanted (Mitsch et al., 1998, 2005a,b). The wetlands receive pumped water continuously from the Olentangy River and can be colonized by aquatic macroinvertebrates from the river that survive the pumps. Surveys of macroinvertebrates have been conducted at the wetland annually, testing a variety of methods (Table 1). In one notable publication, Spieles and Mitsch (2000) reported on intensive sampling of macroinvertebrates in the two created wetlands from April to October 1997 during the wetlands' fourth growing season, using emergence traps and Hester-Dendy samplers. They

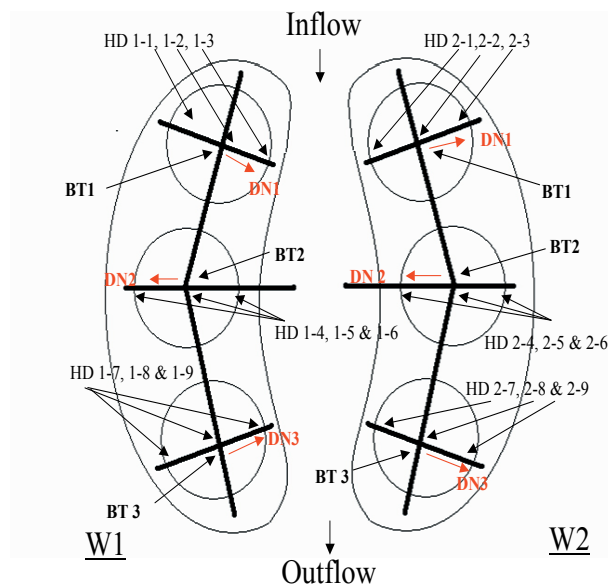


Figure 1. Map of experimental wetland 1 (W1) and wetland 2 (W2) showing locations of Hester-Dendy traps (HD), Bottle traps (BT) and Dipnet samples (DN).

found a total of 39 taxa in the two wetlands and found that average diel dissolved oxygen readings provided the best prediction of invertebrate community metrics.

### Sampling Design

Macroinvertebrates were collected from inflow, middle and outflow regions of W1 and W2 using the three sampling methods described below. The sampling locations were kept consistent with previous years' studies as recommended by Frazier and Mitsch (2000). Hester-Dendy, bottletraps and dipnets were chosen as sampling methods as they have been successfully used before, and capture a diverse range of macroinvertebrates. Sampling locations were stenciled on the boardwalk during this study to facilitate consistent sampling in the future.

### Hester Dendy Plates

Hester-Dendy (HD) plates, constructed of eight 8 x 8 cm masonite plates stacked together, and varying in clearance from 5 to 10 mm, are an artificial substrate sampler (Hester and Dendy, 1962). The advantage of using HD traps is that they are a quantitative sampler. Nine sets of plates were placed in each wetland, three in the inflow region, three in the middle region and three in the outflow as shown in Figure 1. Each set of HD plates was hung with string from the boardwalk, and suspended just above the sediment. The plates were left in place for 15-day periods: Sept. 8-Sept. 23, 2004 ("September sampling") and Oct. 19-Nov. 3, 2004 ("October sampling"). At the end of the sampling period, the plates were removed with care so as not to disturb the samples, and placed in labeled plastic bags. For the September sample, the macroinvertebrates were gently washed with water or scraped from the plates using a spatula into a bucket. The bucket contents were then

sieved through a #40 sized sieve (opening = 420 microns) and backwashed using 95% ethanol into labeled jars that were then topped off with more alcohol to preserve the macroinvertebrates for later identification. For the October sample, the plates were gently washed using 70% ethyl alcohol and the macroinvertebrates were scraped into trays for identification.

### Dipnet

Nets are the most effective way to collect many different kinds of organisms since they can sample surface water, water column, vegetation surfaces, and sediments (Voshell, 2002). Dipnet samples were collected by walking along the boardwalk for two meters, and jabbing a standard D-frame dipnet into the sediment 5 times while sweeping it through the water column. Then the net was swept two meters back through the disturbed area to the starting point to capture dislodged or escaping invertebrates (Acharyya and Mitsch, 2001).

Dipnet samples were emptied into plastic bags in the field and sieved through a #40 sieve in the laboratory. Using a squirt bottle containing 95% ethanol, samples were back-flushed into jars that were filled with additional alcohol and labeled for later sorting and identification of macroinvertebrates. Dipnet samples were collected every two days from Sept. 11-Sept. 23, 2004 (7 times total) from the inflow, middle and outflow regions of each wetland (Figure 1). The density of vegetation in the sampling area was noted for each dipnet sample taken.

### Bottle Traps

Predator macroinvertebrates, such as beetles, are often missed in dipnet samples, because they swim too quickly to be caught and because many are nocturnal. To catch predators, a type of passive funnel trap was used called a bottle trap, as it is made from a 2-L plastic soda bottle (The Volunteer Monitor, 1998). The top of each bottle was cut off at the shoulder and inverted into the base creating a funnel as described by Custer and Johnson (1999). Each bottle was weighted with water and suspended from the boardwalk, with the funnel facing the inflow to facilitate macroinvertebrates swimming into the funnel.

Samples were collected every two days from Sept. 11-Sept. 23, 2004 using one trap in the inflow, middle and outflow of both wetlands as shown in Figure 1. To collect samples, bottle traps were pulled up, the water inside them was poured into plastic bags and the inside of each bottle and funnel was scraped to remove attached snails. Samples were sieved and preserved in alcohol as with the dipnet samples.

### Identification

Invertebrates were identified to the lowest feasible taxonomic level using a dissecting scope. Identifications were based on keys by Merritt and Cummins (1978, 1996), McCafferty and Provonsa (1981), Peckarsky et al. (1990), Thorp and Covich (2001), and Voshell (2002). Insect taxa

Table 2a. Macroinvertebrates colonizing Hester-Dendy plates in September, 2004

CLASS	ORDER	FAMILY	Inflow		Middle		Outflow		Total W1	Total W2	Total all	% of total
			W1	W2	W1	W2	W1	W2				
Gastropoda	Pulmonata	Physidae	32	96	4	12	11	10	47	118	165	11.9
"	Pulmonata	Planorbidae	0	0	0	3	1	4	1	7	8	0.7
"	Right-handed snail	Right-handed snail	1	0	0	1	3	0	4	1	5	0.4
Pelecypoda	Veneroida	Sphaeriidae	1	5	0	10	1	2	2	17	19	1.4
Insecta	Coleoptera	Dytiscidae	0	0	0	0	1	0	1	0	1	0.1
"	Coleoptera	Hydrophilidae	0	1	0	1	6	1	6	3	9	0.6
"	Coleoptera	Elmidae	0	0	0	1	0	0	0	1	1	0.1
"	Diptera	Ceratopogonidae	0	1	3	0	1	1	4	2	6	0.4
"	Diptera	Culicidae	0	2	1	1	3	0	4	3	7	0.5
"	Diptera	Chironomidae	63	56	77	38	179	29	319	123	442	32.0
"	Diptera	Tabanidae	0	0	0	1	0	0	0	1	1	0.1
"	Diptera	Tipulidae	1	0	0	0	0	0	1	0	1	0.1
"	Ephemeroptera	Baetidae	8	8	18	0	93	0	119	8	127	9.2
"	Ephemeroptera	Caenidae	2	0	1	21	51	19	54	40	94	6.8
"	Hemiptera	Corixidae	0	1	2	0	0	0	2	1	3	.2
"	Homoptera	Aphidae	0	2	0	1	0	0	0	3	3	.2
"	Odonata	Aeshnidae	0	0	0	0	3	0	3	0	3	.2
"	Odonata	Coenagrionidae	9	5	0	31	14	11	23	47	70	5.0
"	Odonata	Libellulidae	0	2	0	8	1	4	1	14	15	1.1
Crustacea	Amphipoda	Gammaridae	6	18	0	3	2	9	8	30	38	2.7
"	Cladocera	Cladocera	2	3	1	1	0	0	3	4	7	0.5
Hirudinea	Leech	Leech	38	63	29	71	14	37	81	171	252	18.2
Arachnida	Acariformes	Hydrocaridae	0	1	1	35	5	14	6	50	56	4.1
Oligochaetes	Aquatic worm	Aquatic worm	5	2	9	23	3	6	17	31	48	3.5
Totals			168	146	392	262	392	266	706	675	1381	100

Table 2b. Macroinvertebrates colonizing Hester-Dendy plates in October, 2004

CLASS	ORDER	FAMILY	GENUS	Inflow		Middle		Outflow		Total W1	Total W2	Total all	% of total
				W1	W2	W1	W2	W1	W2				
Gastropoda	Pulmonata	Lymnaeidae	Physa	2	4	0	0	2	0	4	4	8	0.7
"	Pulmonata	Physidae		17	4	19	14	9	31	45	49	94	8.5
"	Pulmonata	Planorbidae		0	0	0	1	0	1	0	2	2	0.2
Pelecypoda	Veneroida	Sphaeriidae	Gammarus	1	0	0	0	0	0	1	0	1	0.1
Insecta	Coleoptera	Dytiscidae		0	0	0	1	0	0	0	1	1	0.1
"	Coleoptera	Hydrophilidae		1	0	0	0	0	0	1	0	1	0.1
"	Coleoptera	Elmidae		2	0	0	0	0	0	2	0	2	0.2
"	Diptera	Chironomidae		18	6	11	2	34	5	63	13	76	6.8
"	Diptera	Tabanidae		3	0	1	0	0	0	4	0	4	0.4
"	Ephemeroptera	Baetidae		0	0	0	2	55	11	55	13	68	6.1
"	Ephemeroptera	Caenidae		0	1	13	86	12	70	25	157	182	16.4
"	Ephemeroptera	Heptageniidae		5	0	0	0	1	0	6	0	6	0.5
"	Hemiptera	Corixidae		1	7	0	2	0	4	1	13	14	1.3
"	Odonata	Aeshnidae		1	1	0	1	1	1	2	3	5	0.4
"	Odonata	Coenagrionidae		48	36	47	16	15	6	110	58	168	15.1
Turbellaria	Tricladidia	Planariidae	Gammarus	6	6	1	9	0	2	7	17	24	2.2
Crustacea	Amphipoda	Gammaridae		76	88	29	134	0	93	105	315	420	37.8
"	Copepoda	Copepods		0	0	0	0	1	0	1	0	1	0.1
Hirudinea	Glossiphoniidae	Leech		22	8	3	0	0	2	25	10	35	3.1
Totals				200	126	130	360	268	226	457	655	1112	100.0

were categorized into one of five trophic functions—collector, piercer, predator, scraper or shredder—according to Merritt and Cummins (1996). Other invertebrates were grouped according to feeding classifications listed in Voshell (2002) and McCafferty and Provonsha (1981).

## Analysis

W1 and W2 were compared to each other in terms of abundance of macroinvertebrates and taxa using a two-way analysis of variance (ANOVA) for the September Hester-Dendy data. Hester-Dendy data were analyzed because this sampling method has been used consistently through the

years. The inflow, middle and outflow regions were also compared using ANOVA. Abundance data were  $\log(X + 1)$  transformed prior to analysis.

The diversity of macroinvertebrates in each wetland was calculated using the Shannon-Wiener function. The Shannon Wiener function ( $H'$ ) is a commonly used diversity index (Hauer and Resh 1996) and is calculated as  $H' = -\sum (p_i \ln p_i)$ .  $H'$  is the index of diversity and  $p_i$  is the proportion of individuals belonging to the  $i$ th species.  $H'$  increases with the number of species in the community, and weights rare species more heavily common ones (Krebs, 1989). For biological communities  $H'$  does not generally go over

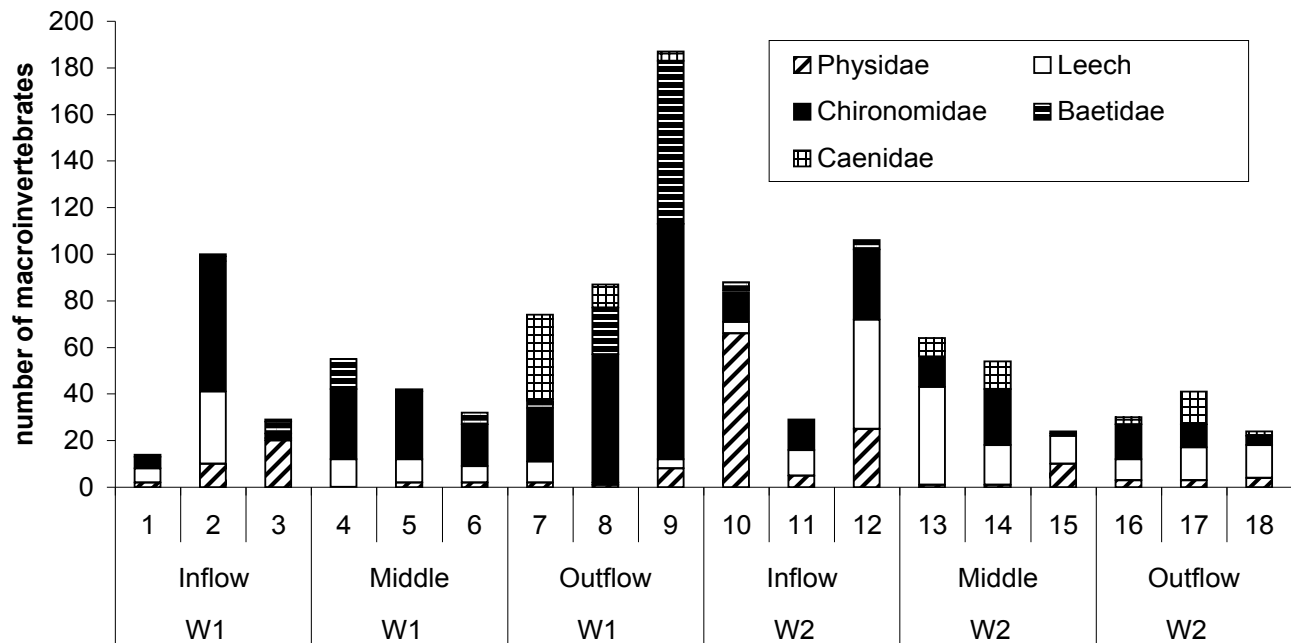


Figure 2. Spatial variation in composition of Hester-Dendy samples in W1 and W2. Top five taxa shown.

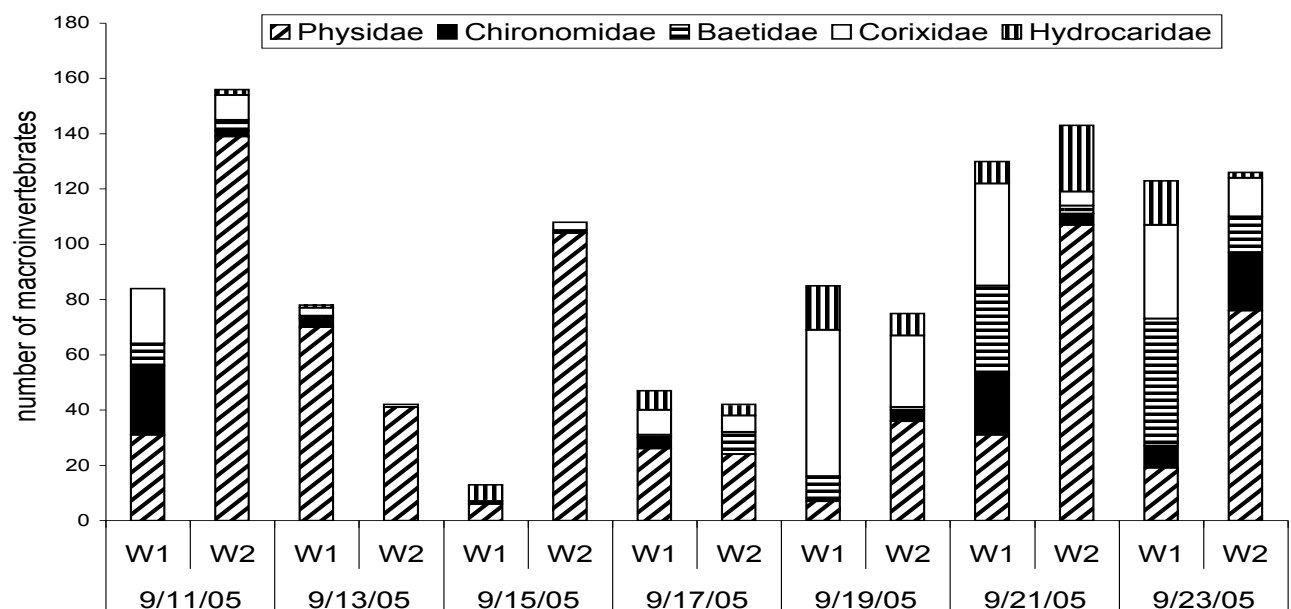


Figure 3. Temporal variation in abundance of macroinvertebrates collected by bottletraps. Top five taxa shown.



5.0 (Washington, 1984). Pielou (1966) advises use of the Shannon-Wiener function only on random samples where the total number of species in the community is known, and recommends the Brillouin index if those conditions are not met. However, Krebs (1989) notes that the results are nearly identical for most ecological samples when the total number of individuals is large. To test for a difference in the Shannon Diversity index between W1 and W2, the Hutcheson t test was used (Hutcheson, 1970; Zar, 1984).

The species evenness of each wetland was calculated using the Shannon Index for Species Evenness (J). The Shannon Index is the most commonly used index of evenness in the literature and is based on the Shannon-Wiener function (Krebs, 1989). J is calculated as:  $J = H' / \ln S$ , where S is the total number of species. J ranges from 0-1, and indicates how evenly distributed individuals are in a habitat.

## Results and Discussion

In the HD samples, the Dipteran family *Chironomidae* was the most abundant, followed by leeches (*Hirudinea*), gastropods (*Physidae*), the Ephemeropterans *Baetidae* and *Caenidae*, and the Odonate *Coenagrionidae*. The remaining taxa accounted for less than 25% of the total (Table 2a). Families *Elmidae* (Coleoptera), *Tabanidae* (Diptera) and *Aphidae* (Homoptera) were not collected in HD samples in W1, while *Dytiscidae* (Coleoptera), *Tilulipidae* (Diptera) and *Aeshnidae* (Odonata) were not found in W2. However, these families were rare, consisting of only 10 organisms total, and some of these families are terrestrial and so may visit the wetlands only temporarily.

A two-way ANOVA on the log-transformed September HD data found no significant difference in abundance of macroinvertebrates between W1 and W2 ( $p = 0.378$ ). However, there was a significant difference in the taxon composition between W1 and W2 ( $p < 0.001$ ). Most of the variation in numbers can be attributed to a few taxa.

The major differences in taxon composition between

W1 and W2 were that the planted wetland (W1) had more *Chironomidae* (319 vs. 123) and *Baetidae* (119 vs. 8), while the naturally colonizing wetland (W2) had more *Physidae* (118 vs. 47), *Coenagrionidae* (47 vs. 23), and leeches (171 vs. 81).

The pattern was similar for the October Hester-Dendy sampling (Table 2b), with W1 having more *Chironomidae* (63 vs. 13) and *Baetidae* (55 vs. 13) and W2 having more *Physidae* (49 vs. 45). However, *Coenagrionidae* and leeches were greater in W1 than W2 (110 vs. 58 and 25 vs. 10, respectively). Another difference in the October sampling was the abundance of the Amphipodan *Gammaridae*. In the September sampling only 8 individuals were collected in W1 and 30 in W2, while the October sampling collected 105 in W1 and 315 in W2, making *Gammaridae* the most abundant taxon. The October sampling may have occurred during a period of *Gammaridae* hatching. Algae were more abundant in W2 than in W1 during the sampling period (Smith et al., 2005), which may explain why there were more of the gastropod *Physidae* in W2 with its larger food base of algae.

The Shannon-Wiener diversity index ( $H'$ ) and Shannon evenness index (J) were computed on Hester-Dendy data (Table 3). For September,  $H'$  was 1.82 for W1 and 2.22 for W2. For October,  $H'$  was 2.06 for W1 and 1.58 for W2, the opposite of September's results. The Hutcheson t-test for difference in  $H'$  revealed a significant difference between the wetlands for the September data ( $p < 0.001$ ,  $W2 > W1$ ). However it also indicated a significant difference for the October data, where  $W1 > W2$  ( $p < 0.001$ ). The diversity indices in 2003 found  $W2 > W1$ . As noted by Frazier and Mitsch (2000), the Shannon index was not meant for taxonomic levels other than species, so the results should be interpreted with caution.

W1, the planted wetland, has generally had greater plant community diversity while the naturally colonizing W2 has been dominated by monocultures of *Typha* spp. and *Schoenoplectus tabernaemontani* (Mitsch et al. 2005b).

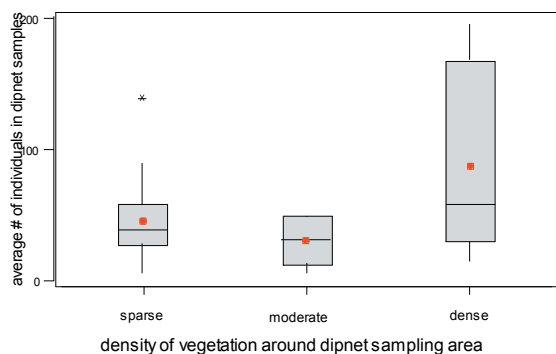


Figure 4. Boxplot of mean number of individuals (dot in box) in 7 samples vs. density of vegetation in area where dipnets samples were taken.

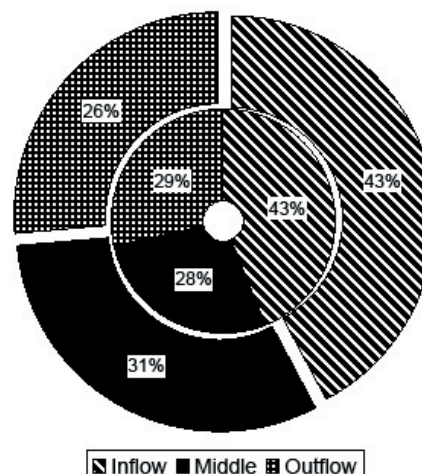


Figure 5. Portion of macroinvertebrates in inflow, middle and outflow of Wetland 1 (inner ring) and Wetland 2 (outer ring) from October 2004 sampling.

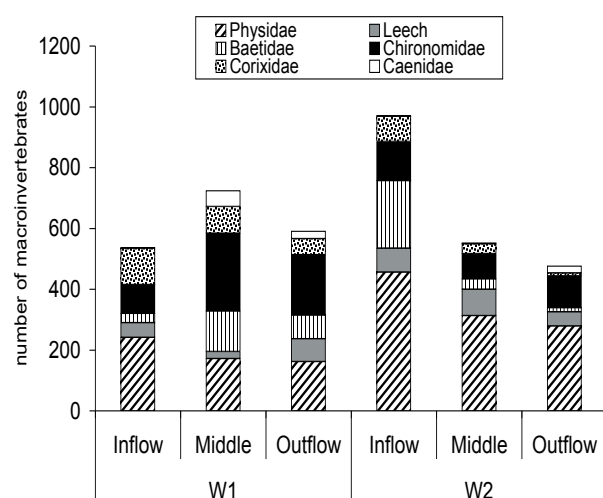


Figure 6. Macroinvertebrates collected in inflow, middle and outflow regions of W1 and W2 by all 3 methods in September. Top 6 taxa shown.

Thus it might be expected that W1 would have greater macroinvertebrate diversity. However, as the wetlands develop, temporal differences in abundance and composition of macroinvertebrates assemblages and spatial patterns of vegetation and alternating open water may be more of a factor than initial plant diversity between the planted and unplanted basins. A study of temporal and spatial patterns of insect emergence from a wetland in Michigan found major *Chironomidae* emergence events occurring in spring/early summer to late summer/early fall (MacKenzie and Kaster, 2004). An emergence event of *Chironomidae* or hatching of other macroinvertebrates, such as *Gammaridae* in October's sampling, could alter the wetland's diversity and evenness and could explain the difference between the September and October results.

There was spatial and temporal variation in abundance of different taxa collected (Figures 2 and 3). Some macroinvertebrate assemblages change seasonally because taxa have been found to colonize wetlands at different times of the year (De Szalay and Resh, 2000). Baetzer and Resh (1992) found temporal changes in abundance and diversity over time in a California wetland due to seasonal egg-laying, fluctuations in water temperature, and fish and waterfowl usage of the wetland. Previous studies have found that there is a good deal of species turnover even in wetlands with permanent water (Jeffries, 1989, 1994).

There was a positive relationship between density of vegetation and abundance of macroinvertebrates in the dipnet samples (Figure 4). The density of submerged vegetation in the sampling area was recorded as either sparse, moderate or dense at the time of the sampling. Analysis with ANOVA found a significant difference between abundance of macroinvertebrates and density of vegetation ( $p=0.016$ ). Plant communities can enhance macroinvertebrate communities by providing food and habitat (Batzer and Wissinger, 1996) as well as structure to the ecosystem,

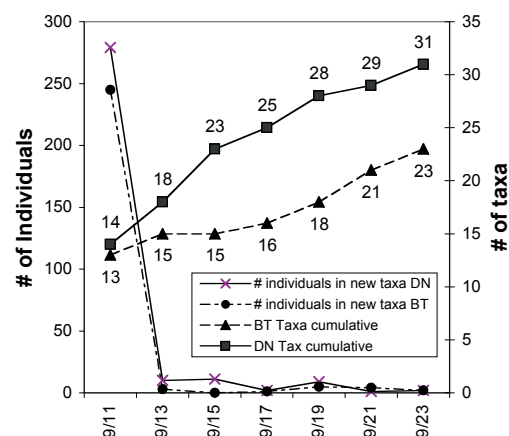


Figure 7. Richness and abundance of macroinvertebrates as sampling effort (number of times sampled) is increased.

altering water flow, enhancing sedimentation rates, creating oxic-anoxic boundaries (Humphrey and Stevenson, 1992), and altering light and temperature by their shading. A study in a constructed marsh in PA found that sites considered to be failures according to invertebrate taxonomic richness also had few macrophytes (Fairchild et al., 1999). A study looking at the influence of emergent plant cover on macroinvertebrate colonization found diversity to be greatest in areas of high plant cover. *Culicidae*, *Ephydriidae* and *Syrphidae* were positively correlated with plant cover while *Corixidae*, *Chironomidae* and *Hydrophilidae* were negatively correlated with vegetation (De Szalay and Resh, 2000).

Previous studies have found a relationship between number of individuals and distance from the inflow (Nairn et al., 1995; Hart et al., 1996; Metzker, 1996), especially for gastropods, which enter and settle out near the inflow of wetlands (Metzker 1996). Nairn (1995) noted that more pollution-tolerant taxa were found near the inflow than pollution-intolerant taxa. October's sampling found more macroinvertebrates in the inflow (Figure 5), and more gastropods were collected in the inflow in the September sampling (Table 2a). The presence of mayflies is in general an indicator of good water quality and there were more of the Ephemeropterans *Baetidae* and *Caenidae* in the outflow of W1. However, the families *Baetidae* and *Caenidae* are exceptions to the rule about mayflies being good indicators of water quality, as they can be found in polluted as well as pristine waters (Voshell, 2002).

A significant difference was found in abundance of macroinvertebrates ( $p=0.032$ ) and in taxon composition ( $p<0.001$ ) between the inflow, middle and outflow regions of W1 and W2 in September. For Hester-Dendy data (Figure 2) the outflow of W1 had the highest abundance, followed by the inflow of W1. When all methods were combined, the highest abundance was in the inflow of W2 (Figure 6). More *Physidae* were collected using the bottle trap and

Table 3. Taxon richness, diversity and evenness within and between W1 and W2 from Hester-Dendy data.

	Wetland 1				Wetland 2			
	IN	MID	OUT	Total	IN	MID	OUT	Total
Species Richness (S) Sept.	12	11	18	21	16	18	13	21
Species Richness (S) Oct.	14	8	9	17	10	11	11	13
Shannon-Wiener (H') Sept.	1.74	1.46	1.67	1.82	1.76	2.19	2.16	2.22
Shannon-Wiener (H') Oct.	1.83	1.61	1.55	2.06	1.44	1.32	1.53	1.58
Evenness (J) Sept.	0.70	0.61	0.58	0.60	0.63	0.76	0.84	0.73
Evenness (J) Oct.	0.69	0.78	0.70	0.73	0.63	0.55	0.64	0.62

Table 4. Summary results of 2004 sampling

Sampling method and date										
	September		September		September		September		October	
	Dipnet		Bottle trap		Hester Dendy		Total all methods		Hester-Dendy	
	# ind.	# taxa	# ind.	# taxa	# ind.	# taxa	# ind.	# taxa	# ind.	# taxa
W1	971	22	653	22	706	21	2330	29	457	17
W2	1061	27	829	16	675	21	2565	30	655	13
Total	2032	31*	1482	23*	1381	24*	4895	35	1112	19

dipnet methods than with the Hester-Dendy traps and it appears that this is why the inflow of W2 had the greatest abundance when these methods were included.

During the study year the hydrology of the wetlands was controlled so that each wetland received a pulse of flooding during the first week of each month. Seasonally-flooded marshes have been found to have greater invertebrate abundance than semi-permanently flooded marshes (Neckles et al., 1990). A study of the Platte River wetlands found that intermediate hydroperiods supported the greatest diversity, while ephemeral sites had reduced biomass and diversity (Whiles and Goldowitz, 2005). There may be some interplay between hydroperiod and predators of macroinvertebrates as invertebrate abundance decreased as pond permanence increased, which may have been due to increased invertebrate and vertebrate predators (Corti et al., 1997).

### Comparisons of Sampling Methods.

A total of 4895 macroinvertebrates were collected from both wetlands in September from a combination of three methods, including 35 taxa representing seven classes and 17 orders (Tables 4 and 5). Eleven additional taxa were collected by adding the dipnet and bottle trap methods to the Hester-Dendy plates. The taxa *Physidae*, *Chironomidae*, *Baetidae*, *Corixidae* and *Hirudinea* occupied the top five positions in the macroinvertebrate community, in that order,

from all three methods. The combined methods yield similar total abundance and richness between W1 and W2, but the taxa and their abundances varied (Table 4 and Appendix A). Dipnets collected the most macroinvertebrates: 2032 individuals as compared to 1482 with bottle traps and 1381 from Hester-Dendy plates. Custer and Johnson (1999) found bottle traps to be the most effective of the three methods, collecting the greatest abundance and diversity of macroinvertebrates, while the second most effective method was the dipnet followed by the Hester-Dendy plates. Acharyya and Mitsch (2001) also found bottle traps to be very effective in collecting a high diversity of macroinvertebrates.

In the bottle trap and dipnet samples, the most abundant organisms were *Physidae* snails (Appendix A). The second most abundant organisms in bottle trap samples were the *Corixidae*. In the dipnet samples, the second most abundant organisms were the *Chironomidae*, followed by the mayfly family *Baetidae*. A combination of all three methods yielded the most common and abundant organisms, *Physidae* snails, which comprised 33% of the samples and were found throughout the wetlands, although their abundance was spatially uneven.

The capturing of some taxa was found to be method specific (Table 5). *Corixidae* are actively swimming organisms and were trapped mainly in the bottle traps. *Aphidae* were collected mainly with dipnets, as were most of the non-aquatic dipterans. The Dipnet captured the most taxa (31), followed by Hester-Dendy (24), and lastly Bottle traps (23, Table 4). The bottle traps captured more beetles and water bugs. Gastropods were the most abundant in bottle traps, making up more than 50% of the sample. More leeches, midges and worms were collected using Hester-Dendy traps and these taxa may be over-represented in samples if only HD data is collected. However Odonates were mainly captured with the HD traps and so these traps are an important collection device, especially with the history of their use at these wetlands.

Selecting a sampling method and determining the number of samples to collect involves trade-offs between the amount of data collected, the taxonomic resolution sought, and the



Table 5. Taxa collected with each method. Shaded cells indicate taxa were not collected by this method.

Guild *	Class	Order	Family	Found with HD	% HD	Found with BT	% BT	Found with DN	% DN	% all methods
SC	Gastropoda	Pulmonata	Physidae	X	11.95	X	48.38	X	36.52	33.18
C	Insecta	Diptera	Chironomidae	X	32.01	X	6.61	X	16.09	17.71
C	Insecta	Ephemeroptera	Baetidae	X	9.20	X	8.43	X	12.70	10.42
PI	Insecta	Hemiptera	Corixidae	X	0.22	X	14.84	X	7.97	7.87
PR	Hirudinea	Arhynchobdellia	Leech	X	18.25	X	4.79	X	1.77	7.33
PR	Arachnida	Acariformes	Hydrocaridae	X	4.06	X	6.34	X	0.59	3.31
SC	Gastropoda	Pulmonata	Planorbidae	X	0.58	X	0.67	X	6.69	3.15
C	Pelecypoda	Veneroida	Sphaeriidae	X	1.38	X	1.21	X	3.54	2.23
C	Insecta	Ephemeroptera	Caenidae	X	6.81	X	0.47	X	0.25	2.17
C	Crustacea	Cladocera	Cladocerans	X	0.51	X	1.96	X	2.95	1.96
C	Oligochaeta	Oligochetes	Aquatic Earthworm	X	3.48	X	0.20	X	2.07	1.90
SC	Gastropoda	Gastropod	Right-handed snail	X	0.36	X	0.88	X	3.30	1.74
C	Crustacea	Amphipoda	Gammaridae	X	2.75	X	1.69	X	0.69	1.57
PR	Insecta	Odonata	Coenagrionidae	X	5.07	X	0.07	X	0.05	1.47
PR	Insecta	Diptera	Ceratopoginidae	X	0.43	X	0.20	X	2.12	1.06
SH	Insecta	Coleoptera	Haliplidae		0.00	X	1.82	X	0.59	0.80
SC	Insecta	Homoptera	Aphidae	X	0.22	X	0.13	X	0.98	0.51
PR	Insecta	Coleoptera	Dytiscidae	X	0.07	X	0.81	X	0.20	0.35
PR	Insecta	Odonata	Libellulidae	X	1.09	X	0.07	X	0.05	0.35
C	Insecta	Coleoptera	Hydrophilidae	X	0.65		0.00	X	0.05	0.20
C	Insecta	Diptera	Culicidae	X	0.51	X	0.07	X	0.05	0.18
SH	Insecta	Hymenoptera	Formicidae		0.00		0.00	X	0.30	0.12
PR	Insecta	Odonata	Aeshnidae	X	0.22	X	0.13	X	0.05	0.12
C	Crustacea	Copepoda	Copepods		0.00	X	0.13		0.00	0.04
C	Insecta	Diptera	Stratiomyidae		0.00		0.00	X	0.10	0.04
SH	Insecta	Diptera	Tipulidae	X	0.07		0.00	X	0.05	0.04
C	Crustacea	Amphipoda	Hyallellidae		0.00		0.00	X	0.05	0.02
C	Crustacea	Ostracoda	Ostracods		0.00		0.00	X	0.05	0.02
PR	Insecta	Coleoptera	Staphylinidae		0.00		0.00	X	0.05	0.02
SC	Insecta	Coleoptera	Elmidae	X	0.07		0.00		0.00	0.02
PR	Insecta	Diptera	Tabanidae	X	0.07		0.00		0.00	0.02
PR	Insecta	Diptera	Helomyzidae		0.00		0.00	X	0.05	0.02
C	Insecta	Hemiptera	Gerridae		0.00		0.00	X	0.05	0.02
PR	Insecta	Hemiptera	Belostomatidae		0.00		0.00	X	0.05	0.02
PR	Insecta	Hymenoptera	Braconidae		0.00	X	0.07		0.00	0.02

\*C = Collector; PR = Predator; PI = Piercer; SH = Shredder; SC = Scraper

considerable time needed to collect, process and identify the samples (Garano and Kooser, 1996). Figure 7 shows the increase in taxa and decrease in number of individuals found in those taxa as sampling effort (number of times sampled) increases. Dipnet samples showed the greatest increase in richness as sampling effort increased. A simple, linear relationship did not exist though, as the rate of new taxa discovered declined over time.

Comparing feeding guilds from Hester-Dendy plates only (Figure 8a) to those from all methods combined (Figure 8b) illustrates that some feeding guilds are better sampled with particular methods, as the combined methods (Figure 8b) led to a much greater representation of scrapers. Both HD and the combined methods showed that collectors were

most abundant in W1 while predators and scrapers were greatest in W2. The abundance of collectors in W1 may inhibit other feeding guilds.

Fish that consume macroinvertebrates can have a negative effect on macroinvertebrate diversity, as some fish may have a preference for certain species or may disturb the sediment with their feeding, reducing macroinvertebrate habitat (Palmer et al., 2000). A study of 11 constructed marshes in Pennsylvania found that the presence of fish had the greatest effect of any habitat variable on the invertebrate community, with invertebrate biomass being four times greater in wetlands with few or no fish (Fairchild et al., 1999).

The bottle trap samples collected 30 fish and 11 tadpoles.

Table 6. Macroinvertebrate diversity at experimental wetlands 1 and 2 from 1994 to 2004 (after Grubh and Mitsch, 2003)

<i>Class</i>		1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	9/2004	10/2004
Arachnida			✓	✓							✓	✓	
Crustacea		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Gastropoda		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Hirudinea				✓	✓	✓	✓	✓		✓	✓	✓	✓
Insecta		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Nematoda (Phylum)										✓			
Oligochaeta		✓		✓	✓	✓	✓	✓		✓	✓	✓	
Pelecypoda (Bivalvia)			✓		✓	✓	✓	✓		✓	✓	✓	✓
Turbellaria			✓										✓
<i>Order</i>	<i>Family</i>												
Acariformes	Hydrocaridae		✓								✓	✓	
Amphipoda										✓			
Amphipoda	Gammaridae	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓	✓
Amphipoda	Hyalloidea	✓	✓	✓	✓	✓	✓	✓	✓		✓		
Arhynchobdellia	Hirudinidae					✓	✓	✓		✓	✓		
Arhynchobdellia	Glossiphoniidae												✓
Cladocera												✓	
Cladocera	Daphnia		✓	✓	✓			✓	✓		✓		
Coleoptera		✓	✓	✓		✓	✓	✓	✓		✓		
Coleoptera	Dytiscidae										✓	✓	✓
Coleoptera	Elmidae										✓	✓	✓
Coleoptera	Halophilidae									✓	✓	✓	
Coleoptera	Hydrophilidae									✓	✓		✓
Coleoptera	Staphylinidae											✓	
Collembola				✓									
Copepoda												✓	✓
Veneroida	Sphaeriidae											✓	✓
Diptera		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Diptera	Ceratopogonidae										✓	✓	✓
Diptera	Chironomidae									✓	✓	✓	✓
Diptera	Culicidae										✓	✓	✓
Diptera	Helomyzidae											✓	✓
Diptera	Stratiomyidae											✓	✓
Diptera	Syrphidae									✓			
Diptera	Tabanidae											✓	✓
Diptera	Thaumaleidae										✓		
Diptera	Tipulidae										✓	✓	
Ephemeroptera		✓	✓	✓	✓	✓		✓		✓	✓		
Ephemeroptera	Baetidae	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓	✓
Ephemeroptera	Caenidae	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓	✓
Ephemeroptera	Ephemerellidae	✓	✓	✓	✓	✓	✓	✓	✓		✓		
Ephemeroptera	Heptageniidae												✓
Hemiptera		✓	✓	✓	✓	✓		✓	✓	✓	✓		
Hemiptera	Belostomatidae											✓	
Hemiptera	Corixidae									✓	✓	✓	✓
Hemiptera	Gerridae												
Hemiptera	Nepidae										✓		
Homoptera	Aphidae											✓	
Hymenoptera									✓				
Hymenoptera	Formicidae											✓	
Hymenoptera	Braconidae											✓	
Lepidoptera											✓		
Odonata		✓	✓	✓	✓	✓	✓		✓		✓		
Odonata	Aeshnidae									✓	✓	✓	✓
Odonata	Coenagrionidae									✓	✓	✓	✓
Odonata	Cordulidae										✓		
Odonata	Libellulidae									✓	✓	✓	✓
Ostracoda												✓	
Plesiopora		✓		✓	✓						✓		
Pulmonata	Lymnaeidae									✓		✓	✓
Pulmonata	Physidae									✓		✓	✓
Pulmonata	Planorbidae									✓		✓	✓
Tricladida	Planariidae									✓			✓
Tricoptera										✓			

Figure 8a and 8b.

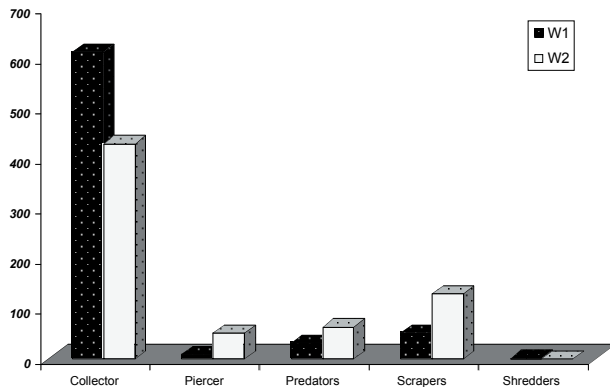


Figure 8a Representation of feeding guilds in W1 vs. W2 from Hester-Dendy data only

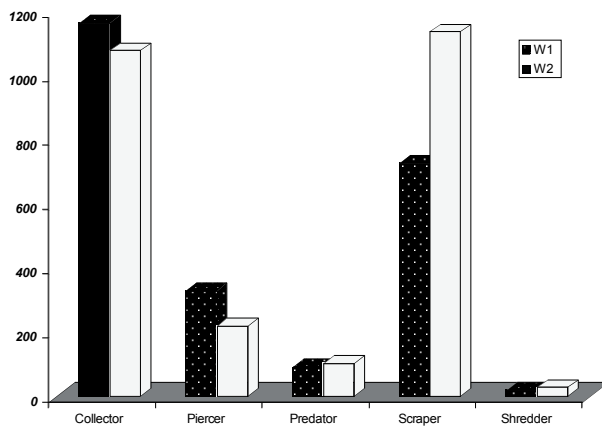
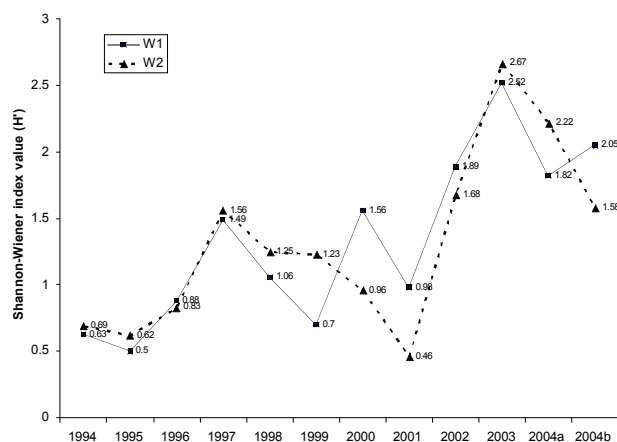


Figure 8b Representation of feeding guilds in W1 vs. W2 from all methods

Figure 9. Shannon-Wiener diversity index for macroinvertebrates in W1 and W2 over 10-year period. Years 1994-1996 from Mitsch et al. (2005a); years 1997-2001 from Webb and Mitsch (2002); year 2002 from Holland and Mitsch (2003) and year 2003 from Grubh and Mitsch (2004).



These vertebrates most likely consumed some of the macroinvertebrates captured in the bottle traps. The bottle trap data were also confounded by the fact that in the Sept. 13 sample the traps in W2 either separated from their funnel or were found floating on top of the water. In spite of this sampling problem in W2, more macroinvertebrates were collected by bottle trap in W2 than in W1. Finally, the effect of bottle trap sampling on non-target organisms should be considered. Eighteen of the vertebrates were found dead when the traps were pulled every other day.

## Comparisons with Previous Studies at the ORW

Summary of results from benthic macroinvertebrate surveys from 1994 to 2004 are given in Table 6. Changes in the Shannon-Wiener diversity indices over the past 10 years are shown in Figure 9. Dodson and Lillie (2001) found a significant positive correlation between zooplankton community taxon richness and number of years since wetland restoration. From their regression results, they estimate a time frame of 6.4 years for wetland sites to recover from agricultural disturbance.

Diversity in the ORW experimental wetlands shows a trend of increase with time. However, sampling noise due to differing methods, sampling effort and sampling size (see Table 1), as well as spatial and temporal variations make one cautious about making conclusions. Each study's differing taxonomic resolution makes comparing Shannon-Wiener indices especially problematic as it was designed for species-level comparison. Also, some studies have used sieves to filter organisms for preservation and identification while other studies have hand-picked organisms from samples. The latter studies may be missing some of the smaller and less visible macroinvertebrates, which may explain lower diversity in some sampling years.

In the last few surveys and this year's in particular there were more taxa of terrestrial insects such as *Aphidae*, *Formicidae*, non-aquatic Dipterans and Hymenopterans than in previous years. Terrestrial invertebrates are important ecologically in wetlands, although most wetland invertebrate research has focused on the aquatic species (Sharitz and Batzer, 1999). Aphids and collembolans were found to be the most abundant invertebrates on the surface of *Potamogeton* (pondweed) beds (Bergey et al., 1993). Batzer and Wissinger (1996) note that terrestrial insects such as aphids, leaf hoppers, moth and butterfly larvae, and beetle larvae and adults are the most important invertebrate consumers of vascular plants in wetlands. Their appearing in samples from W1 and W2 may indicate that the wetland has matured to the point of supporting a terrestrial community large enough to be sampled.

## Conclusions

Thirty-five taxa were found between the two wetlands this

year (2004) using three collection methods in September. Most of those taxa were found in small numbers, with 20 taxa contributing less than 1% to the total number of organisms. In both wetlands, the total number of individuals as well as the number of taxa were similar. However, the taxa and their abundances differed between wetlands, with large temporal and spatial variations. W2 had more algae in the fall than W1 which may explain the greater abundance of gastropods in this wetland, which feed on algae. W2 also had more predators and scrapers than W1, while W1 had more collectors. The abundance of collectors in W1 may inhibit other taxa. Although sampling methods and efforts have varied through the years, the cumulative data do appear to indicate that the diversity of macroinvertebrates has generally increased in these wetlands over time.

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## Appendix A.

Method	Hester Dendy Traps						Bottle Traps						Dipnet							
Location	Inflow		Middle		Outflow		Inflow		Middle		Outflow		Inflow		Middle		Outflow			
Taxa	W1	W2	W1	W2	W1	W2	W1	W2	W1	W2	W1	W2	W1	W2	W1	W2	W1	W2	Total	% of total
Hydrocaridae	0	1	1	35	5	14	17	1	31	8	6	31	3	0	3	4	0	2	162	3.31%
Aquatic Earthworm	5	2	9	23	3	6	0	1	0	0	2	0	1	3	16	6	14	2	93	1.90%
Physidae	32	96	4	12	11	10	141	253	29	90	20	184	69	199	132	127	130	85	1624	33.18%
Planorbidae	0	0	0	3	1	4	2	1	3	1	2	1	3	3	37	19	62	12	154	3.15%
Sphaeriidae	1	5	0	10	1	2	3	1	3	3	3	5	9	6	15	12	11	19	109	2.23%
R--handed snail	1	0	0	1	3	0	0	1	1	0	9	2	0	5	10	20	25	7	85	1.74%
Leech	38	63	29	71	14	37	3	43	3	12	1	9	7	7	6	12	3	1	359	7.33%
Gammaridae	6	18	0	3	2	9	0	3	0	0	8	14	0	13	0	0	0	1	77	1.57%
Cladoceran	2	3	1	1	0	0	2	1	7	4	11	4	2	56	0	0	2	0	96	1.96%
Hyalllellidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0.02%
Copepods	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	2	0.04%
Ostracods	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0.02%
Dytiscidae	0	0	0	0	1	0	4	1	1	3	0	3	1	1	0	1	0	1	17	0.35%
Elmidae	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0.02%
Haliplidae	0	0	0	0	0	0	4	5	5	2	0	11	1	0	7	2	0	2	39	0.80%
Hydrophilidae	0	1	0	1	6	1	0	0	0	0	0	0	0	0	0	0	1	0	10	0.20%
Staphylinidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0.02%
Ceratopoginidae	0	1	3	0	1	1	0	0	1	0	2	0	1	0	23	2	15	2	52	1.06%
Chironomidae	63	56	77	38	179	29	1	9	9	5	56	18	31	43	68	22	105	58	867	17.71%
Culicidae	0	2	1	1	3	0	0	0	0	0	1	0	0	0	0	0	0	1	9	0.18%
Tabanidae	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0.02%
Tipulidae	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	2	0.04%
Helomyzidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0.02%
Stratiomyidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	2	0.04%
Baetidae	8	8	18	0	93	0	11	14	18	12	67	3	11	190	22	14	11	10	510	10.42%
Caenidae	2	0	1	21	51	19	0	0	0	3	2	2	0	1	1	0	2	1	106	2.17%
Corixidae	0	1	2	0	0	0	101	43	24	15	31	6	19	38	64	16	21	4	385	7.87%
Belostomatidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0.02%
Gerridae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0.02%
Aphidae	0	2	0	1	0	0	0	0	0	0	2	0	0	11	0	7	2	0	25	0.51%
Formicidae	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	4	0	1	6	0.12%
Braconidae	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0.02%
Aeshnidae	0	0	0	0	3	0	0	0	0	0	2	0	0	0	0	0	1	0	6	0.12%
Coenagrionidae	9	5	0	31	14	11	0	0	1	0	0	0	0	0	1	0	0	0	72	1.47%
Libellulidae	0	2	0	8	1	4	0	0	0	1	0	0	0	0	0	0	1	0	17	0.35%
Total individual	168	266	146	262	392	147	290	377	136	159	227	293	159	577	405	270	407	214	4895	100
Total taxa	12	16	11	18	18	13	12	14	14	13	19	14	14	15	14	17	17	22	35	

